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Phenotypic and environmental factors associated with elevated autoantibodies at clinical onset of paediatric type 1 diabetes mellitus

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ABSTRACT

To examine possible determinants of autoantibody levels at type 1 diabetes mellitus (T1DM) onset.

We assessed levels of glutamic acid decarboxylase 65 islet cell antigen (GADA) and anti-insulin antibodies (IAA) in 247 incident T1DM cases presenting <15 years of age in Melbourne from 1st March 2008 to 30th June 2010.

58.9% (142/241) of cases were GADA seropositive and 42.3% (94/222) were IAA seropositive. Factors associated with elevated IAA antibodies included younger age and red hair phenotype. Factors associated with elevated GAD antibodies included lower birthweight and recent eczema. Intriguingly, low recent or past sun exposure was only associated with elevated GADA levels among children presenting at age <5 years, not older (difference in effect, $p<0.05$ for 4 of 5 associations).

These findings show that environmental and phenotypic factors are associated with autoantibody levels at time of presentation for T1DM. We recommend such environmental and phenoytypic factors should be examined in further detail.

1. Introduction

Type 1 diabetes mellitus (T1DM) is increasing among children worldwide [1]. In Australia, the incidence has increased by 3% annually between 2000 and 2006 [2]. In Victoria, Australia and many locations, the incidence increase is greater among children under 5 [3,4]. These children often have a more severe clinical presentation, are less likely to have a honeymoon period in their subsequent course and are more likely to be destined to have complications by adulthood because of a prolonged disease duration [5].

The majority of early onset T1DM have evidence of diabetes associated autoantibodies (AA) at first presentation, reflecting pancreatic beta-cell destruction, accompanied by an autoimmune inflammatory response within the pancreatic islets ('insulitis'). Diabetes autoimmunity occurs when autoantibodies to islet antigens (such as antibodies against insulin or glutamic acid decarboxylase 65), are produced before the onset of clinical disease [6]. The risk of progression to clinical diabetes increases with the number of autoantibodies detected [7]. Two important antibodies are those against the glutamic acid decarboxylase 65 islet cell antigen (GADA) and those directed against insulin (IAA). The formation of insulin antibodies is an early event in diabetes autoimmunity. Several large and well designed cohort studies are now underway to assess the determinants of diabetes autoimmunity and T1DM. Observational cohorts of high risk individuals have now demonstrated that transient diabetes autoimmunity occurs more commonly than persistent diabetes autoimmunity [8]. A 2011 report comparing two similar birth cohorts from 1989 and 2000 found a similar level of diabetes autoimmunity in both cohorts but higher clinical T1D incidence in the 2000 cohort, concluded that "accelerated progression from islet autoimmunity to diabetes is causing the escalating incidence of type 1 diabetes in young children" [9].

An assessment of AA levels at T1DM onset can provide insights into the possible determinants and influences on AA generation among children who are destined to develop T1DM. It has been known for more than thirty years that HLA-linked genetic control influences IAA immunity [10]. Studies have reported that IAA are associated with class I HLA-B and HLA-C types [10], and GADA levels have been positively associated with select class II HLA

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alleles and haplotypes [11]. Other studies have reported on how AA levels vary among incident T1DM cases by age, sex and body mass index (BMI) or disease characteristics such as diabetic ketoacidosis [5,12,13].

However, relatively few studies have reported on the association between past and current environmental factors or child phenotype and the profile of AA levels among T1DM children at disease onset. In Australia, child onset T1DM incidence from 1999 to 2004 was characterised by an overrepresentation of winter diagnoses and an inverse trend with ambient ultraviolet radiation (UVR) levels for low population density regions, where ambient UVR was postulated to better reflect child personal UVR dose [14]. We, and others, have reported fair skin pigmentation to be overrepresented among T1DM cases [9,15,16]. Other factors such as recent infection, diet and family history are also of interest.

Here, we aim to investigate the factors independently associated with higher IAA and GADA levels at T1DM onset among a clearly defined sample of children residing in the Melbourne area who presented with T1DM during 2008 to 2010 with a special focus on children presenting with T1DM at age <5 years.

2. Materials and methods

2.1. Subjects

This report is based on incident T1DM cases recruited between March 2008 and June 2010 at the Diabetes Services, Royal Children's Hospital, Melbourne, Australia and the Paediatric Diabetes Services, Monash Children's, Monash Medical Centre, Melbourne (Latitude 38°S), Australia. These are the two tertiary referral centres for children who develop T1DM and reside in the Melbourne Metropolitan area. Case ascertainment has been ascertained by capture – recapture methods to be 98% [3]. Children and their families were either interviewed during the first inpatient admission or at the following outpatient clinics thereafter. Inclusion criteria were incident cases of T1DM presenting at these two clinical centres age 1–14 years inclusive. Exclusion criteria were if an infant presented with diabetes under the age of one year, severe congenital abnormality or disease, particularly those leading to a lack of usual school attendance. The study interview was conducted by trained interviewers and involved a parental questionnaire and clinical examination of the child. Parents were asked about the amount of time their child would normally have spent in the sun during weekends and holidays in winter and summer at various ages using questions with demonstrated validity [17]. Child time in the sun has been previously associated with late winter serum 25 hydroxyvitamin D (25OHD) levels: winter weekends ($r=0.23$), winter weekdays ($r=0.17$) and winter school holidays ($r=0.22$) [18]. Winter weekend sun-exposure (<2 h, 2–3 h, 3–4 h, >4 h) in healthy girls in an adjacent Australian state also correlated with lumbar spine density ($r=0.29$, $p=0.002$) [19]. A comprehensive infection, demographic, lifestyle and child environment history was also recorded [15].

Written consent was obtained from parents and also from children 12 years of age and older. Ethical approval was obtained from the Human Research Ethics Committee of the Royal Children's Hospital, Melbourne, Australia and the Human Research Ethics Committee of Monash Medical Centre, Melbourne, Australia.

2.2. Examination data

The research nurse noted the natural skin and eye colour (with reference to standardized colour photographs) and ethnicity (White [listed as "Caucasian" on the examination form], Asian, African, Australian Aboriginal or Torres Strait Islander or Middle Eastern), and undertook a naevi count on the left arm [15,20]. Skin reflectance on the buttock (a non-sun exposed site), left back shoulder, upper inner arm and hand were measured at wavelengths 400 and 420 nm using a hand-held spectrophotometer (Minolta 2500d) to estimate cutaneous melanin density [21]. Previously, these spectrophotometric measurements have been shown to be correlated ($r=0.68$) with the histological measurements of cutaneous melanin [21]. Other past sun markers such as silicone rubber impressions of the dorsum of both hands [22] and ultraviolet fluorescence photography [23] were also collected but are part of an ongoing study and have thus not yet been analysed.

2.3. Laboratory data

Overall, for the 328 eligible children who presented with T1DM from 1st March 2008 to 30th June 2010, IAA and GADA AA levels were determined at the time of admission. GADA was measured by precipitation of in vitro transcribed-translated GAD65 biosynthetically labelled with [^{35}S]methionine. Sensitivity and specificity in the Diabetes Autoantibody Standardisation Programme [24] was 94% and 76%, respectively. IAA was measured by precipitation of ^{125}I-(A14) human insulin [25]. Sensitivity and specificity of the IAA assay in the Diabetes Autoantibody Standardisation Program was 22% and 99%, respectively. The thresholds for GADA and IAA positivity, 5 and 1.0 units/mL, respectively, were established as the 97.5 percentiles of unselected healthy children and young adults [26]. IAA and GADA levels were determined at the Royal Melbourne Hospital laboratory, Melbourne, Australia. The laboratory is a Nationally Accredited Testing laboratory for these tests. AA to GADA was available for 97.6% (241/247) of participants and IAA was available for 89.9% (222/247) of participants. The mean time between date of first presentation to hospital and the date of environmental examination was 6.4 (SD 6.8) weeks.

2.4. Statistical methods

The distribution of each AA level was first examined. The distributions were strongly skewed and thus a logarithmic transformation was used before further analysis. As there were a number of AA levels of zero, half the next lowest value was added to the zero's to enable all the results to be logged. Cut-off points for BMI for overweight and obese classification by age and sex were adopted from UK based criteria for children [27]. BMI scores under the overweight cut-off point were considered normal. Data were obtained for the monthly average of daily total UVR dose in Standard Erythemal Doses for Melbourne from 1993 to 2010 [28]. The UVR at month of birth was the average of UVR for the month for the years 1993–2008. This was the range of birth years for the cases. The UVR for the month of diagnosis was the monthly UVR assessed at the month and year of diagnosis. UVR was similarly determined for the child's 12th week of gestation only for Victorian born infants. The total UVR at 12 weeks pregnancy was obtained by multiplying the number of hours spent in the sun by the hourly UVR dose. Because the biological effect of sun exposure depends not only on time in sun but also the amount of skin exposed and whether exposed skin has a sunscreen barrier, we derived a composite sun exposure index, based partly on the total sun exposure score of Hanwell et al., which has been shown to account for 38% of variance ($p=0.002$) in serum 25OHD for adults at a latitude of 40°N [29]. A composite child sun exposure index last summer/winter was created. Children with greater time in the sun, less clothing, and where data available, less sunscreen were given a higher score (Supplementary Tables).

Multiple linear regression was used to examine how factors related to continuous AA levels. We were also interested to examine the presence of any AA and how factors may, in particular, predict multiple AA. To examine factors associated with seropositivity to both IAA and GADA, adjusted odds ratios and 95% confidence intervals were estimated by logistic regression. For multivariable analyses using either of these models, potential confounders such age and sex were included in the models as covariates to provide adjusted estimates of mean difference or risk, respectively. We used Stata 11.1 software (StataCorp, College Station, TX) for all analyses.

3. Results

The case sample consisted of 247 children under 15 years of age who were diagnosed at the two Melbourne hospitals from 1 March 2008 to 30 June 2010. The overall case participation rate was 75.3% (247/328). The case sample was gender balanced with 47.4% (117/247) females and a mean age at diagnosis of 8.3 (SD 3.7) years. Almost all the participants were Caucasian, 90.2% (222/246), and the skin pigmentation of the buttock was 2.5% melanin (SD 1.5). Nearly one fifth (19.0% (47/247)) of the cases presented by age <5 years (Fig. 1). GADA seropositivity was evident in 58.9% (142/241) of participants and IAA in 42.3% (94/222) of participants. The majority of those seropositive for IAA (≥ 1.0 units/mL) also had evidence of GADA seropositivity (≥ 5 units/mL): 62.4% (58/93). Overall, 48.9% (108/221) were seropositive to one antibody and 26.2% (58/221) were positive to two antibodies. Among those with multiple antibodies the quantitative titre of antibodies was only weakly correlated ($r=0.13$ (-0.001 to 0.26), p-value$=0.05$).

3.1. Factors associated with either IAA or GADA antibody levels.

In general, parental characteristics were not associated with GADA levels at presentation (Table 1). The following factors were not associated with either GADA or IAA antibody levels – maternal exposure to cats or dogs or maternal farm residence in pregnancy.

Table 1 reports associations between child characteristics and GADA or IAA antibodies. Older age was associated only with lower IAA levels, with a halving of the IAA level for every four year increase in age. IAA antibodies were positively associated with darker skin pigmentation (Fig. 2). The geometric means (95% CI) of GAD antibodies by skin type were:-fair skin, 5.05 (2.41, 10.59); medium/fair skin, 5.67 (3.68, 8.73); olive/medium skin, 3.54 (1.75, 7.18); olive skin, 10.93 (4.09, 29.20) and dark skin, 13.81 (2.21, 86.18). Although mean GAD antibody levels were highest for the two categories of darker skin, an overall trend by skin type category was not evident ($p=0.44$). In contrast, the geometric means (95% CI) of IAA antibodies by skin type were as follows: fair skin, 0.88 (0.50, 1.53); medium/fair skin, 0.68 (0.51, 0.91); olive/medium skin, 0.94 (0.65, 1.35); olive skin, 1.53 (0.64, 3.67) and dark skin, 3.93 (0.01, 10.99). After adjustment for child age and sex, the increase in IAA level by darker skin type category remained significant ($p=0.048$). After adjustment for child age and sex, a family history of diabetes (insulin dependent diabetes mellitus, non insulin dependent diabetes mellitus or diabetes/gestational diabetes) and darker skin pigmentation were positively associated with IAA levels and these factors were taken into account in subsequent analyses. Maternal smoking in pregnancy and current paternal smoking were not associated with GADA or IAA levels.

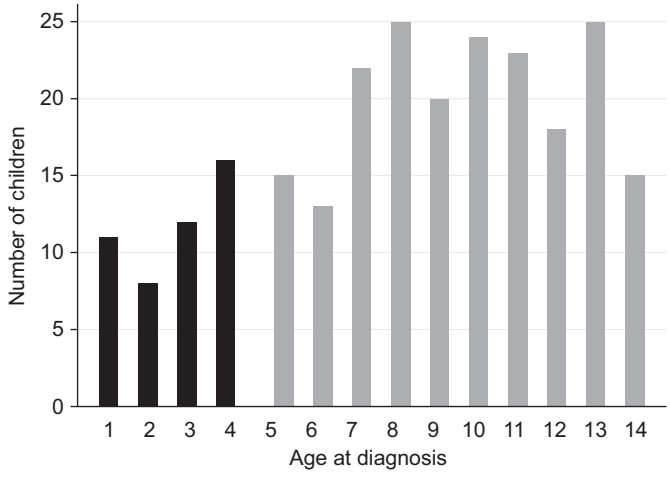

Fig. 1. Age of onset distribution of type 1 diabetes mellitus cases presenting during 2008–2010 in Melbourne, Australia.

Children who were reported to have had a cold or flu-like illness or a urinary tract infection in the three months prior to diagnosis had higher IAA antibody levels than children who had not. AA levels were not associated with asthma or hay fever history but children reported to have had eczema over the past 12 months had five-fold higher GADA levels (Table 1). There was no association between total, older or younger sibling number, or body mass index or child weight with AA levels. Here, maternal vitamin D supplementation and fish consumption tended to be associated with slightly higher, not lower GADA levels but it must be remembered that in Australia UVR is the major determinant of vitamin D status [30]. Only one child consumed fish oil supplements so these could not be formally examined.

3.2. Logistic regression to identify factors associated with multiple autoantibodies

We examined how child characteristics predicted the likelihood of having both antibodies detected vs. either no antibodies or just one antibody (Table 2). The likelihood of seropositivity to both AA declined with age ($p=0.02$). Again, skin pigmentation appeared important with each 1% increase in melanin density associated with an odds ratio of 1.39 or 1.43 for multiple AA seropositivity vs. no antibodies or one AA, respectively. Older or younger sibling number, past sun exposure, fish consumption, recent (within the previous 3 months) infection history and a history of asthma, hay fever or eczema over the past year were not associated with multiple AA seropositivity. Children with a past history of any ear infection were more likely to exhibit multiple AA seropositivity and a history of chicken pox was associated with a reduced likelihood of multiple vs. no AA seropositivity.

3.3. Age at onset

We further examined if case characteristics differed by age of onset. Unsurprisingly, younger children were less likely to report a range of infections and to have younger mothers and fathers at age of diagnosis. Cases presenting at age <5 years had higher IAA levels but not GADA levels than cases presenting at an older age (IAA geometric mean for young diagnosed 2.89 (95% CI 1.69 to 4.95), for older diagnosed 0.64 (95% CI 0.52 to 0.80), $p<0.001$; GADA geometric mean for young diagnosed 3.02 (95% CI 1.37 to 6.64), for older diagnosed 6.16 (95% CI 4.46 to 8.51), $p=0.07$). Individual adjustment for red hair, skin colour, buttock melanin density, obesity, urinary tract infection in the past 3 months and a history of lice in the past 3 months did not alter the evidence for excess IAA levels among the younger group.

Table 1
Associations between parent and child characteristics and (log-scale) GADA and IAA levels at presentation.

	GADA levels ($n=241$)			IAA levels ($n=222$)		
	Geometric mean ratios[a]	95% CI	p value	Geometric mean ratios[a]	95% CI	p value
Parental age at diagnosis						
Maternal age at diagnosis (per year)	0.99	0.92, 1.06	0.68	1.01	0.96, 1.05	0.77
Paternal age at diagnosis (per year)	0.98	0.92, 1.04	0.50	1.02	0.98, 1.06	0.32
Antenatal smoke exposure						
Mother smoked during pregnancy (per increase in categories[b])	0.70	0.44, 1.14	0.16	0.93	0.68, 1.27	0.64
Mother ever smoked during pregnancy	0.42	0.16, 1.09	0.08	0.75	0.40, 1.42	0.38
Father smoked during pregnancy (per increase in categories[b])	1.00	0.76, 1.31	0.99	0.94	0.78, 1.14	0.54
Father ever smoked during pregnancy	0.62	0.30, 1.31	0.22	0.84	0.51, 1.39	0.51
Maternal sun exposure						
Total ambient UVR levels at 12 w pregnancy during weekdays (Standard erythemal doses per one unit increase)	0.94	0.76, 1.14	0.52	1.10	0.97, 1.24	0.15
Total ambient UVR levels at 12 w pregnancy during weekends (standard erythemal doses per one unit increase)	0.95	0.79, 1.14	0.56	1.11	0.99, 1.24	0.07
History						
Mother took vitamin D supplements during pregnancy	6.08	0.92, 40.06	0.06	2.66	0.79, 9.05	0.12
Family history of diabetes (yes vs. no)	1.76	0.94, 3.28	0.08	1.06	0.70, 1.61	0.78
Child examination[c]						
Hair colour (red vs. other)	2.32	0.59, 9.08	0.23	2.77	1.13, 6.80	0.03
Eye colour (blue/green vs. other)	0.87	0.47, 1.63	0.67	0.81	0.53, 1.24	0.33
Buttock melanin density (per 1% increase in melanin density)	1.09	0.89, 1.35	0.41	1.19	1.03, 1.36	0.02
BMI categories[a]						
Normal weight	Ref.	–	–	Ref.	–	–
Overweight	1.04	0.49, 2.22	0.92	1.13	0.68, 1.89	0.64
Obese	2.40	0.59, 9.87	0.23	1.15	0.42, 3.18	0.79
Child history						
Age	1.06	0.97, 1.15	0.19	0.84	0.80, 0.90	<0.001
Male	1.01	0.54, 1.90	0.96	1.03	0.68, 1.57	0.90
Birthweight (per kg)	0.51	0.30, 0.86	0.01	1.02	0.72, 1.46	0.89
Gestation (per week)	0.87	0.74, 1.03	0.10	0.98	0.88, 1.09	0.69
Sun exposure						
Composite time in sun during last summer weekends (low to high sun exposure)	0.94	0.82, 1.07	0.34	0.97	0.88, 1.06	0.47
Composite time in sun during last winter weekends (low to high sun exposure)	1.05	0.89, 1.23	0.59	0.97	0.87, 1.08	0.56
Composite time in sun during last summer holidays (low to high sun exposure)	0.91	0.80, 1.04	0.18	0.94	0.86, 1.02	0.14
Composite time in sun during last winter holidays (low to high sun exposure)	1.03	0.88, 1.19	0.73	0.92	0.84, 1.02	0.11
Fish consumption						
Oily fish consumption, categories[d]	2.33	1.17, 4.64	0.02	0.76	0.47, 1.21	0.25
Oily fish eaten at least once a week	2.48	1.14, 5.38	0.02	0.75	0.43, 1.28	0.29
Infections and atopy						
Influenza or cold in the past 3 months	0.96	0.50, 1.82	0.89	1.52	1.00, 2.34	0.05
Ear infection in the past 3 months	1.87	0.50, 7.03	0.36	1.90	0.75, 4.83	0.18
Urinary infection in the past 3 months	0.26	0.00, 27.64	0.57	32.46	1.65, 634.76	0.02
Lice in the past 3 months	0.82	0.30, 2.24	0.70	0.54	0.28, 1.04	0.07
Asthma in the past 12 months	0.62	0.20, 1.99	0.43	0.93	0.45, 1.94	0.84
Asthma in the past 12 months among those with asthma ever	0.41	0.08, 1.95	0.27	1.14	0.36, 3.58	0.82
Eczema in the past 12 months	4.99	1.04, 23.94	0.046	2.37	0.81, 6.90	0.12
Hay fever in the past 12 months	1.32	0.39, 4.44	0.66	1.16	0.53, 2.56	0.71

[a] Adjusted for age at diagnosis, sex, buttock melanin density and family history of diabetes.
[b] Increasing categories; nil, less than daily, 1–10 cigarettes per day, 11–20 cigarettes per day, 21–40 cigarettes per day.
[c] Adjusted for age at diagnosis, sex and family history of diabetes only in the child examination section.
[d] Increasing categories; never or occasionally, once or twice per week, three or more times a week.

3.4. Sun exposure

T1DM cases who were reported to spend less than one hour outside during the last winter non-school days also had higher ($p=0.02$) GAD antibody titres (adjusted mean difference 3.64 fold (95% CI 1.23, 10.75)) international units than cases who spent more time outside. Table 3 examines the association between sun exposure and GAD antibody levels by age of diagnosis. Among T1D cases presenting at age <5 years, there is a consistent pattern that those with higher sun exposure over the past summer or winter weekends or holidays had lower GADA levels after adjusting for age, sex and skin pigmentation. However, among children diagnosed from age 5 and older, a consistent pattern was not seen. Consistent with the above, the report of having spent less than an hour in the sun in summer or winter weekends or holidays at ages 0–2 years was associated with increased GADA levels only for children presenting at age <5 years. The difference in the apparent effect of past sun exposure on GADA levels by age of T1D onset was significant ($p<0.05$) for 4 of the 5 associations examined (Table 3).

4. Discussion

A high prevalence of GAD antibody positivity and, to a lesser extent IAA positivity was evident among T1DM cases at initial presentation. IAA positivity was associated with younger age. GADA levels were associated with a reduction of UVR levels in the

winter prior to presentation, particularly for children presenting at age <5 years.

Because of the marked increase in T1DM incidence among children at age <5 years in our location [3], this report has a substantial proportion of very young (aged <5 years) incident T1DM cases. Here, we demonstrated that these incident cases have higher IAA levels but were not able to explain the higher IAA levels in terms of the recognised measured differences between these younger presenting cases and those aged 5–14 years. Younger cases also tended to have higher GADA levels and this could partly be accounted for by a greater adverse apparent effect of low past child sun exposure on GADA levels among children at age <5 years at presentation.

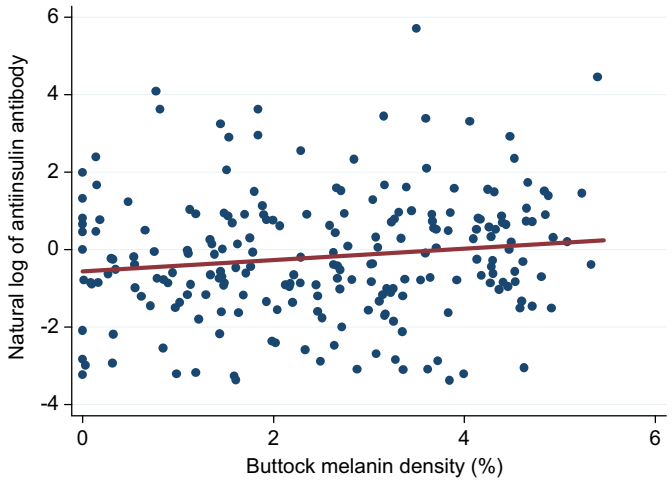

Fig. 2. Increased skin melanin density is associated with higher IAA. Age and sex adjusted test for trend; p-value=0.01. R square for full model; 14.5%.

Previously, fair skin type has been reported to be more common among children with T1DM compared to controls [31] and siblings [15]. Here, IAA levels at T1DM onset were higher for children of darker pigmentation and was also higher among children with red hair. This finding in relation to phenotype may reflect underlying genotype in that HLA genotypes vary by race and have previously been associated with antibody levels at T1DM onset [11,32]. It may also reflect related UVR and vitamin D metabolism genes. We have previously shown that the association between vitamin D receptor gene and T1D onset varies by regional UVR levels [33]. The accelerator hypothesis postulates that the more obese child should develop T1DM at a younger age and that higher insulin levels linked to insulin resistance may underlie the formation of insulin AA [34]. Further, β cell upregulation in response to obesity-linked insulin resistance may lead to upregulated cellular enzymes and associated antibodies such as

Table 2
Odds ratios for the development of both GADA and IAA vs. no autoantibodies or one antibody.

	Both AA detected (GADA and IAA) vs. none[a]			Both AA detected (GADA and IAA) vs. one[a]		
	OR	95% CI	p value	OR	95% CI	p value
Child examination[b]						
Hair colour (red vs. other)	4.64	0.46, 46.74	0.19	1.34	0.35, 5.06	0.67
Eye colour (blue/green vs. other)	0.83	0.37, 1.87	0.65	0.55	0.27, 1.12	0.10
Buttock melanin density (per 1% increase in melanin density)	1.39	1.04, 1.88	0.03	1.43	1.12, 1.83	0.004
History						
Age at diagnosis	0.87	0.77, 0.98	0.02	0.92	0.84, 1.01	0.08
Male	0.94	0.41, 2.15	0.87	0.73	0.35, 1.51	0.40
Family history of diabetes	1.68	0.74, 3.81	0.22	1.35	0.66, 2.76	0.41
Older siblings (0, 1, 2, 3 or more)	1.20	0.76, 1.91	0.44	1.40	0.95, 2.06	0.09
Younger siblings (0, 1, 2, 3 or more)	0.62	0.33, 1.17	0.14	0.66	0.39, 1.14	0.14
Influenza or cold in the past 3 months	1.25	0.52, 2.98	0.62	1.03	0.50, 2.11	0.94
Ear infection ever	3.28	1.32, 8.13	0.01	2.44	1.15, 5.18	0.02
Chicken pox ever	0.38	0.14, 1.02	0.06	0.77	0.34, 1.74	0.53

[a] Adjusted for age at diagnosis, sex, buttock melanin density and family history of diabetes.
[b] Adjusted for age at diagnosis, sex and family history of diabetes only in the child examination section.

Table 3
The difference in sun's effect on GADA levels by age of diagnosis.

	Child under 5 years			Child 5–14 years			p value for difference in effect by age
	Geometric mean ratios[a]	95% CI	p value	Geometric mean ratios[a]	95% CI	p value	
Composite time in sun during last summer weekends (low to high sun exposure)	0.58	0.38, 0.86	0.01	0.99	0.86, 1.14	0.92	0.01
Composite time in sun during last winter weekends (low to high sun exposure)	0.56	0.36, 0.89	0.02	1.17	0.99, 1.38	0.07	0.002
Composite time in sun during last summer holidays (low to high sun exposure)	0.75	0.52, 1.07	0.12	0.93	0.81, 1.08	0.35	0.28
Composite time in sun during last winter holidays (low to high sun exposure)	0.74	0.50, 1.11	0.15	1.11	0.95, 1.31	0.20	0.04
Under 1 hour spent in sun during summer weekends/holidays at ages 0–2	5.82	0.93, 36.53	0.07	0.89	0.44, 1.81	0.75	0.04

[a] Adjusted for sex, buttock melanin density and family history of diabetes.

GADA [34]. However, here, children under 5 were not heavier than older T1DM children. Also, no link between obesity and autoantibody levels at T1DM diagnosis were observed.

One prospective cohort study has demonstrated that mothers who do not take vitamin D supplementation in pregnancy or have lower vitamin D stores in pregnancy have offspring who are more likely to develop AA by age 3 years [35]. However, no associations were observed in the Finnish Diabetes Prediction and Prevention study [36]. In Australia, vitamin D stores are predominantly derived from radiation exposure to the skin. Here, lower sun exposure in the winter prior to presentation was associated with elevated GAD antibodies at onset.

Intriguingly, the association between low early life sun exposure and elevated GADA antibodies was more evident among children present with T1DM onset at age <5 years, indicating that low early life UVR exposure or low vitamin D stores may play a particular role in the generation of GAD antibodies among children who develop T1DM by age 5 compared to those who present with T1DM at older ages. However, as this is the first report of such an association, replication of these findings are required. The finding of effect modification by age of onset, together with the findings in the Swedish cohort that maternal vitamin D supplements were associated with GADA at 1 year but not at 2.5 years [35] indicate that further analysis of the Finnish cohort [36] is warranted to examine whether the null findings overall mask a difference in the effect of maternal vitamin D status by age of advanced autoimmunity or T1DM onset.

Strengths of this study includes that it is on a well defined population within a specific geographical area. Further this study examined not only phenotype factors but also environmental factors taking into account potential confounding effects of age, sex and family history of diabetes. Limitations of this study include that it is based on cases only, limiting causal inference and data on HLA type, a well- known associate of autoantibody levels, was not available. However, collection of control data is shortly due for completion. Here, maternal vitamin D supplementation and fish consumption tended to be associated with slightly higher, not lower GADA levels but it must be remembered that in Australia UVR is the major determinant of vitamin D status.

Some intriguing patterns were evident in relation to early life sun exposure. Among young cases (<5 years of age) lower reported recent (over the past year) or past (at ages 0–2 years) sun exposure was associated with higher GADA levels, a pattern that generally differed significantly to the associations evident for T1D cases present at or above five years of age.

It has been proposed for T1DM that early life microbial exposure may have both an adverse and also beneficial effect [37,38]. Here, a history of an upper respiratory tract infection, influenza or urinary tract infection was associated with higher IAA levels, consistent with a possible short term immunity boosting effect of infection, through bystander activation or other mechanisms [39]. A longer term influence of factors is suggested by the finding that children with a history of any ear infection were more likely to have multiple antibodies. Further work is needed to determine whether this marker was evident by chance or reflects an impaired host response to infection rather than ear infection also [40].

In conclusion this study suggests environmental factors such as sun exposure may have an influence on the development of antibodies in childhood T1DM particularly during the early years of life. This is a critical developmental phase for adaptive immunity. These findings show the potential importance of environmental and phenotypic, as well as genetic, associations with autoantibody levels at time of presentation for T1DM presenting under the age of 15 years. As some of our findings were novel, they require replication in future studies of T1DM.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.rinim.2012.06.002.

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